

### **REMARKS**

Claims 1-19 are pending and claims 20-51 have been withdrawn subject to an earlier restriction requirement. No claims have been allowed. Upon entry of the amendments, claims 1-15 and 17-18 will be pending, of which claims 1 and 17 have been amended. Claim 1 has been amended to include the elements of claim 16 and to indicate that the detected species is a sulfonyl halide derivative of a sulfonylhydrazone of a ketosteroid. Claim 17 has been amended without prejudice to update its dependency from claim 16 to claim 1. Support for these amendments is provided in the claims as originally filed as well as in paragraphs [0137] – [0144] and Figs. 8A-D. Applicants reserve the right to file the original scope of the claims in one or more continuing applications. No new matter is entered upon entry of these amendments.

#### **Rejections of the Claims Under 35 U.S.C. § 103**

##### **Visser in view of Bailey**

Claims 1-9 and 12-14 stand rejected under 35 U.S.C. § 103 for allegedly being obvious over Visser et al. (“Visser”) in view of Bailey et al. (“Bailey”). Applicants traverse the rejection on the grounds that the alleged combination of Visser and Bailey fails to provide all of the elements of Applicants’ invention as claimed. For example, claim 1, as presently amended, requires that the sample is reacted with a sulfonyl halide following reacting the sample with the sulfonylhydrazide. Because neither Visser nor Bailey discloses reacting a sample with a sulfonyl halide following reacting the sample with the sulfonylhydrazide, Applicants submit that the rejection should be withdrawn.

##### **Visser in view of Bailey**

Claims 1-15 stand rejected under 35 U.S.C. § 103 for allegedly being obvious over Berliner in view of Bailey et al. (“Bailey”). Applicants traverse the rejection on the grounds that the alleged combination of Berliner and Bailey fails to provide all of the elements of Applicants’ invention as claimed. For example, claim 1, as presently amended, requires that the sample is reacted with a sulfonyl halide following reacting the sample with the sulfonylhydrazide. Because neither Berliner nor Bailey discloses reacting a sample with a sulfonyl halide following reacting the sample with the sulfonylhydrazide, Applicants submit that the rejection should be withdrawn.

**Visser in view of Bailey, or Berliner in view of Bailey, and further in view of Takadate**

Claims 16-18 stand rejected under 35 U.S.C. § 103 for allegedly being obvious over Visser in view of Bailey, or Berliner in view of Bailey, and further in view of Takadate et al. (“Takadate”). Applicants traverse the rejection on the grounds that the invention is not obvious as the cited prior art does not teach or suggest to one of ordinary skill in the art to react a ketosteroid with a sulfonylhydrazide to form a sulfonylhydrazone **and subsequently** to react the resulting sulfonylhydrazone with a sulfonyl halide. The Office Action at page 5, lines 4-10 states that

“Takadate et al. teach that derivatization of ketosteroid estrogens using a sulfonyl halide is an alternative derivatization to that of ketosteroids using sulfonylhydrazides. It would have been obvious to one of ordinary skill in the analytical chemistry arts *to follow* the derivatization of ketosteroids using sulfonylhydrazides with an *alternative* and well-known method of derivatization of ketosteroids using sulfonyl halide. *The purpose or advantage of doing so would be to check the relative accuracy of the first method.*” (emphasis supplied)

Applicants respectfully submit that this reasoning is incorrect. In fact, the purpose or advantage of reacting a ketosteroid with a sulfonylhydrazide to form a sulfonylhydrazone and subsequently reacting the resulting sulfonylhydrazone with a sulfonyl halide is quite different than that stated in the Office Action. As well, Applicants have observed surprising results in carrying out their method. According to the specification at paragraph [0138], with combined sulfonylhydrazide derivatization of the carbonyl and sulfonyl derivatization of the hydroxyl (e.g., as exemplified in Applicants’ Example 8 with TSH and dansyl chloride, respectively), all estrogens and estrogen metabolites show intense protonated molecule  $[MH^+]$  and less abundant natriated molecule  $[MNa^+]$  during ESI positive ion mode. In contrast, some D-ring ketolic estrogen metabolites, such as 16 $\alpha$ -hydroxyestrone, 16-ketoestradiol, 2-hydroxyestrone, and 4-hydroxyestrone, show rather poor response and hydrogen loss when derivatized with the dansyl chloride procedure alone without prior carbonyl protection (**FIG. 8**), which is believed to be due to their alkali labile nature. The extensive hydrogen loss found in deuterium-labelled D-ring ketolic estrogen metabolites with dansyl chloride derivatization alone is especially problematic since ion clusters from their

protonated or natriated molecules also cover those of target analytes (**FIGS. 8 and 9**), which makes accurate quantitative measurement difficult.

Unlike the dansyl chloride derivatization alone, the combined TSH carbonyl protection and dansyl chloride derivatization is a method suitable for the quantitative measurement of all endogenous steroids (such as estrogens and estrogen metabolites) by HPLC-ESI-MS. (Specification, paragraph **[0138]**).

Hence, Applicants' claimed two-step process of carbonyl derivatization followed by hydroxyl derivatization provides for better HPLC separation of steroids, and allows for better signal detection in API-MS (such as ESI-MS) when at least one of the derivatization groups contains a highly ionizable moiety that enables ionization under either positive ion or negative ion mode of electrospray ionization. Because neither Visser in view of Bailey, and further in view of Takadate, nor Berliner in view of Bailey, and further in view of Takadate discloses reacting a sample with a sulfonyl halide followed by reacting the sample with the sulfonylhydrazide *in order to improve signal detection in API-MS*, Applicants submit that the rejection should be withdrawn.

For all of these reasons, Applicants submit that the claims as presently amended should be allowed and urge the examiner to issue a Notice of Allowability as soon as possible. If there are any questions, the examiner is invited to contact the undersigned attorney at the telephone number listed below.

Respectfully submitted

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